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OF ITS DERIVATIVES

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UNIVERSITY OF CALIFORNIA PUBLICATIONS IN AGRICULTURAL SCIENCE

Volume 6, No. 9, pp. 231-255, 4 figures in text

Issued February 6, 1932

UNIVERSITY OF CALIFORNIA PRESS

BERKELEY, CALIFORNIA

CAMBRIDGE UNIVERSITY PRESS

LONDON, ENGLAND

THE INTERSPECIFIC HYBRID, *CREPIS RUBRA* X *C. FOETIDA*, AND SOME OF ITS DERIVATIVES.

II. TWO SELFED GENERATIONS FROM AN AMPHIDIPLOID HYBRID

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A plant of F_1 *Crepis rubra* x *C. foetida* was isolated from other *Crepis* species in 1927. At maturity the achenes were gathered, and in the following year these gave rise to nine F_2 plants, among which were found 3 diploids, 5 triploids, and 1 amphidiploid. The latter proved to be the first *Crepis* hybrid of this type to exhibit any degree of fertility, possessing 2.7 per cent of the estimated potential number of achenes. Demonstration that new species may arise in *Crepis* through amphidiploidy would prove of great interest, since all but two of the American *Crepis* species occur in chromosome numbers with a base number of 11, a situation which is presumably due to amphidiploidy. Other chromosome numbers in *Crepis* have been assumed to arise through amphidiploidy (Babcock & Navashin, 1930), and the discovery of an amphidiploid with such low fertility was therefore disappointing, although grounds existed for believing it could give rise to fertile and stable recombination products.

From among the rapidly increasing number of amphidiploids in the literature, several indicate sterility of the same order of magnitude as that found in *Crepis rubra-foetida*. One of them, *Nicotiana rustica-paniculata* (Lammerts 1931), has produced four distinct derivative lines, which may indicate similar potentialities in some, if not all, partly sterile amphidiploids. Discovery of stable derivative lines in a *Crepis* hybrid of this type would present certain advantages not to be expected in any amphidiploid thus far known. First, the chromosome numbers of the two parents are considerably lower than in any other known amphidiploid and, second, the somatic chromosome

garniture shows five different morphological types, of which three kinds indicate parental origin.

Furthermore, it was hoped that study of the progeny of this hybrid might throw some light on certain limits encompassing gametogenesis and fertilization. A study of meiosis in this hybrid showed striking irregularities in the pollen mother cells (Poole 1931) at diaphase, first and second metaphase, and the anaphases. The differences clearly showed that it was impossible to distinguish meiotic units as to their valences, since the number of units observed varied from the minimum expected number of quadrivalents, five, to as high as thirteen. It was impossible to say which were univalents of large homologues, quadrivalents of small ones, or any other possible valences, since the range in number indicated the probable presence of all types. It is therefore pointless to attempt examination of meiotic stages in derivative plants until some fairly stable individuals are found. Progress thus far has indicated that derivatives with strikingly different somatic chromosome garnitures, when used as parents will assort at random, producing offspring in the next generation with a wide range in somatic garnitures.

In the following investigation plants of two selfed generations from the F_2 amphidiploid hybrid are studied. Cytological examination was confined to somatic cells in root tips fixed in Navashin's solution and stained with Haidenhein's haemotoxylin. The chromosome garnitures of metaphase plates were drawn with a Spencer camera lucida, at a magnification of 3700. The following drawings are reproduced without reduction. The number of cells studied for each plant varied with the nature of the chromosome rearrangements and the spacing of the chromosomes. *Crepis* species are excellently adapted for such studies, since any root tips that are properly fixed and stained readily reveal any number of reliable plates. Furthermore, advantage was taken of M. Navashin's method of imbedding a half-dozen root tips in a single block.

THE F_3 GENERATION

The first selfed generation, F_3 , of the amphidiploid plant comprised 45 plants, of which 44 reached maturity, and permitted collection of some interesting data. In regard to chromosome number these 45 plants grouped themselves as follows:

$4n-1$ (19)	$4n$ (20)	$4n+1$ (21)	$4n+2$ (22)
11	23	10	1
	10 with no rearrangements 13 with rearrangements		

Roughly speaking one-fourth of the plants lack one chromosome, and in these plants the absent chromosome may be any one of the twenty comprising the amphidiploid garniture; or any two chromosomes may be lacking, but with the substitution of a third. One-half of the

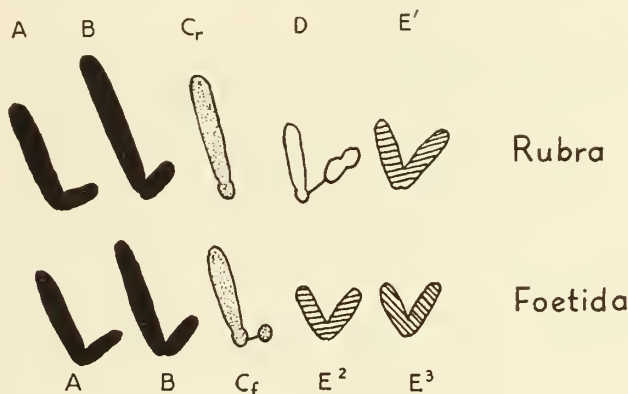


Fig. 1. Illustrating a haploid genom from each parent and showing the five different kinds of chromosomes in the amphidiploid garniture.

population had the full tetraploid number, and of these half appeared to retain the same amphidiploid garniture as the parent, while the remaining half exhibited various rearrangements, involving substitutions of one, two, or three homologues. Finally one-fourth possessed one chromosome extra (in one case two extra); but the extra number was seldom due to the simple addition of a chromosome; rather the additions involved substitutions often as complex as in either of the preceding classes.

Considering the amphidiploid genom as a unit, we may recognize five distinct types of chromosomes, as in figure 1.

The distinction between A and B is not a convenient one to follow, and the four members of each will be referred to as 8A. Two E's are contributed by *rubra*, and four by *foetida*. It is assumed from previous studies that two of these E's from *foetida* conjugate with the highly individual pair of D's from *rubra*, but it is impossible to decide from the total number of six E's which two are concerned. The C chromosomes, however, are clearly differentiated, the two from *rubra* being longer and without satellites, whereas the two from *foetida* are shorter and possess good sized spherical satellites. The C's are never confused with the D's, because the body of the latter is one-half the length of the body of the former and the satellites are not only many times the size of the C satellites, but always appear to have a double nature. This readily recognized amphidiploid set may then be described as $8A\ 2C_r\ 2C_f\ 2D$ and $6E$, and for convenience is simply designated $4n$. In the subsequent tabulations of data from F_3 and F_4 the chromosome garnitures will be indicated as $4n$ plus or minus the appropriate chromosomes.

A fairly complete tabulation of F_3 is given as table 1, in which only two morphological characters are included, since the other dozen characters used in the study lacked correlation with any useful data.

Chromosome garnitures.—The 45 plants comprising the F_3 population are arranged in table 1 according to (a) chromosome numbers and (b) the complexity and relationship of their rearrangements. The somatic garniture is represented by a descriptive formula in the right hand column.

An inspection of table 1 discloses a number of valuable facts as to (a) the fertility of certain somatic garnitures and (b) the relation of the two character pairs, nodding-erect buds before anthesis and purple-yellow anther tubes, to the cytological evidence of the presence of the chromosomes in which their genes are believed to have their loci.

In regard to fertility a great disparity exists between plants in the euploid and the aneuploid groups. Nearly all the plants in the euploid group exhibit some degree of fertility, whereas all the plants in the aneuploid group are sterile or practically so, with two exceptions. The relative magnitude of the percentages of good pollen grains in the two groups is equally marked. The correlation between pollen grains and fertility has been treated in a former paper (Poole 1932) but in that paper the complete figures for F_3 were not presented as they are here in table 1. Furthermore, among the euploid plants the

TABLE 1
GENETIC AND CYTOLOGICAL DATA OF F₃
20 CHROMOSOME PLANTS

Plant No.	Bud position	Anther tubes	Per cent good pollen	Per cent fertility	No. of achenes	Formulae
B 3	nodding	tipped	22.7	0.35	10	} 4n
8	erect	tipped	33.7	0.30	7	
9	nodding	tipped	28.0	3.45	90	
12	nodding	tipped	37.0	fertile	?	
16	nodding	tipped	19.4	0.35	12	
C 3	nodding	tipped	22.2	0.59	17	
7	nodding	tipped	35.3	2.22	66	} -D+E
10	nodding	tipped	31.2	0	0	
14	nodding	tipped	22.4	1.26	45	
17	nodding	tipped	33.9	2.73	59	
A 1	nodding	tipped	33.3	1.85	30	} -C _r +C _t
B 7	nodding	tipped	56.2	6.71	109	
C 15	erect	tipped	47.0	1.93	45	
22	nodding	tipped	42.1	0.07	2	
B 13	nodding	tipped	34.4	4.08	97	} -C _r +C _r
17	erect	tipped	31.5	0.56	7	
C 13	nodding	tipped	38.2	0.91	22	} -2C _t +2C _r
20	nodding	purple	19.2	0.77	15	
C 9	nodding	tipped	17.3	0	0	} -C _t +C _r -D+A
B 18	nodding	tipped	42.4	8.56	158	
C 12	nodding	tipped	29.5	0	0	} -C _t +C _r -D+E
16	nodding	tipped	39.5	0.10	3	
B 6	nodding	purple	17.4	0	0 -C _r +C _t -E+D

19 CHROMOSOME PLANTS

B 1	nodding	tipped	31.6	0	0 -C _r +C _t -D
C 11	nodding	yellow	17.7	0	0 -2C _r +2C _t -D
B 19	nodding	tipped	27.2	2.13	59	} -2C _r +C _t
C 19	nodding	yellow	17.6	0	0	
2 -C _r +C _t -A
B 20	erect	tipped	23.0	0	0 -C _t +C _r -D
15	nodding	tipped	15.8	0	0	} -C _r -D+E
22	nodding	tipped	30.8	0.31	12	
2	erect	tipped	12.1	0	0	} -D
10	nodding	tipped	23.9	0	0	
14	erect	tipped	40.7	0.01	2	

21 CHROMOSOME PLANTS

B 4	nodding	tipped	20.4	0	0	} -2D+2E+C _t
5	erect	tipped	13.7	0	0	
C 8	nodding	tipped	16.1	0.35	9	} -2D+2E+A
21	nodding	tipped	28.4	0.04	1	
23	nodding	tipped	25.6	0.43	18 +D
C 1	nodding	tipped	20.2	0	0	} +A
5	nodding	tipped	14.9	0	0	
6	nodding	tipped	22.2	0.50	11	
18	nodding	tipped	26.7	0.28	4	
B 11	nodding	tipped	21.4	1.27	56	

22 CHROMOSOME PLANTS

B 21	nodding	tipped	26.3	0	0 +A+E
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seven sterile or practically sterile plants are mainly confined to that group at the bottom of the 20-chromosome group in which rearrangements involve more than one pair of homologous chromosomes. Two plants of this group C 9 and B 6, possess one chromosome each unknown to either parent species. C 9 had a chromosome resembling a *rubra*-D with either a duplication or a translocation attached, and B 6 had two normal D's and a third chromosome looking like a normal D minus the large double satellite. The garnitures of both plants are illustrated in figures 2a and 2b.

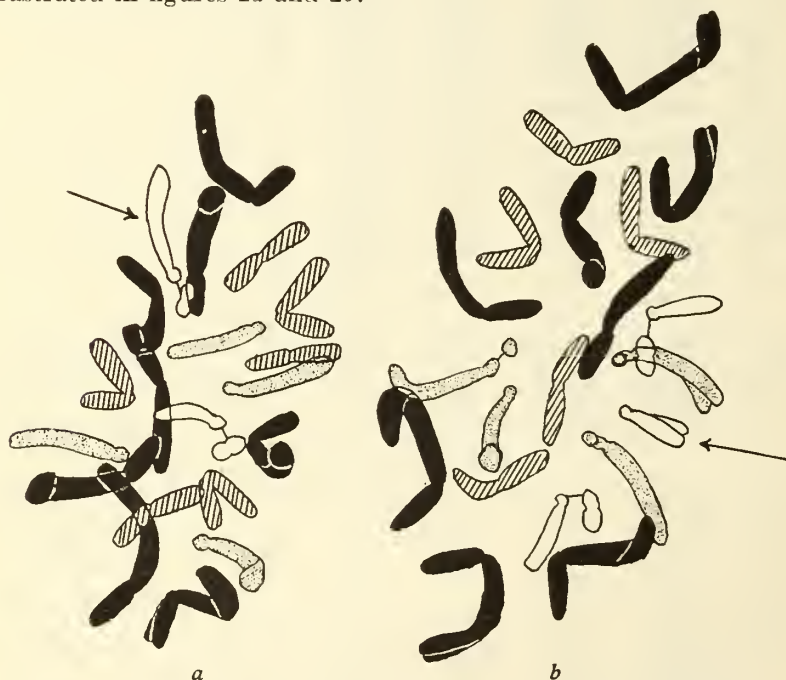


Fig. 2. Two garnitures of F_3 plants each showing new chromosomes as a result of alterations in the D chromosomes: (a) C 9 with a garniture of $4n-2C_r+2C_r$, and one of the D's possesses an attachment; (b) B 6 with a garniture of $4n-C_r+C_r-E+D$, and one D lacks its satellite.

The most fertile group of plants is that from A 1 to C 20, in which chromosomal rearrangements involve a single pair of homologues. This situation raises the hope that further improvement in fertility lies in the progeny of such plants. One exception exists to the observation that rearrangement in one pair of homologues enhances fertility beyond the amphidiploid set, and that is B 18, the most fertile of the population. The exceptional nature of this plant will be discussed later, because it is not only exceptional among the F_3 population, but also the F_4 , one-half the plants of which were derived from it.

Some significance may be attached to the fact that aneuploid plants are found in the progeny of an amphidiploid. No aneuploid plants have ever been found among derivatives of the cross *C. rubra* \times *C. foetida* having a diploid number of chromosomes, whereas aneuploids are as numerous as euploids among tetraploid derivatives. Probably functional gametes are restricted in number to $2n \pm 1$, although it is possible that the number 22 was obtained from $10 + 12$ or $9 + 13$. At any rate, the variation in chromosome number of functional gametes in *rubra-foetida* is much more restricted than that found in functional gametes of hybrids in which the somatic number is greater. Hence the reaction system of a plant with smaller chromosome numbers is more susceptible to losses or gains than the reaction system of a plant with higher numbers, as shown previously in *Datura* tetraploids by Belling and Blakeslee (1924).

Considering the exceptional nature of B 18, it is probable that the chromosome rearrangement is no greater than that found in plants involving only a single pair. The chief distinction between the C chromosomes of the two parents rests on the presence or absence of the satellite. In certain backcross plants of ($F_1 \times rubra$) \times self certain exceptions to expected results point to the possibility of interspecific crossing over. The somatic garniture of plant B 9 (fig. 3c) as well as B 18 (fig. 3a) may represent the effects of crossing over. In both plates one of the chromosomes designated *foetida* C is too long for a normal C_t chromosome, despite the fact that a *foetida* satellite is present. The length of the C chromosomes in these two cases suggests that of C_r chromosomes. In B 9 the satellited chromosome in the center of the plate is too long for C_t and the so-called C_r immediately to its right is entirely too short for a *rubra* C chromosome.

The question arises, is this cytological evidence of crossing over, or merely a result of fragmentation and attachment? The observed chromosome alterations may conceivably represent the former situation, because single cross overs between these two interspecific homologues would be expected to produce such alterations.

Consequently, it is probable that the third C_r chromosome of B 18 is partly or mainly *foetida* in constitution. Furthermore, the apparently extra A chromosome in the same plate could easily be an E chromosome with an attached segment, hence, indistinguishable from an A. There are good reasons for supposing this to be true, as will be discussed presently. In other words, it is possible that B 18 belongs genetically in that group of *euploid* plants possessing rearrangements in only one set of homologues.

Plant C 10 is the only plant classified as $4n$ which proved unable to set seed, and since it had 31.2 per cent good pollen grains, and seemed in other respects to be an amphidiploid, its sterility was



Fig. 3. (a) B 18, most fertile plant of F_3 with a garniture of $4n-Cr+Cr-D+A$ but with one so-called Cr too long for a normal Cr , (b) B 7 the second most fertile F_3 plant with garniture $-D+E$ (both B 7 and B 18 were used as parents for F_4); (c) B 9 classified tentatively as a $4n$ plant but with abnormal Cr and Cr chromosomes.

probably due to some non-genetic causes. This conclusion is strengthened from a consideration of B 12, also a $4n$ plant with no rearrangements, which had 37 per cent good pollen, and which was observed

to have set seed before an untimely death. Therefore, it may be considered that the only sterile *euploid* plants in F_3 are those in which rearrangements involve more than one set of homologous chromosomes. On the other hand, rearrangements in the eight A and six E chromosomes are undetectable, and if sterility in plants with only one rearrangement is accompanied by low pollen grain counts, such undetectable rearrangement in A's or E's could be inferred; e.g., B 16 and C 20.

Evidently departures from the amphidiploid constitution are limited in range if fertility is to be maintained, since it appears that the loss or gain of a single chromosome, or the rearrangement of more than a single pair of homologues contributes to both genetic and zygotic sterility. The question then arises, to what extent may improvements in the fertility of amphidiploids be expected where all the homologues from both genoms are capable of quadrivalent conjugation?

Genetic analysis.—The second feature of interest from the data of table 1 concerns the factor pairs nodding-erect bud position, and purple-yellow anther tubes. In a previous publication (Poole 1931) it was shown that the characters, nodding buds and purple anther tubes, come from *rubra*, and the characters, erect buds and yellow anther tubes, come from *foetida*. In the backcross of F_1 to *rubra* × self the combined genetic and cytological analysis showed the following association between chromosomes and characters:

C_rC_r nodding buds, purple anther tubes

C_rC_r nodding buds, purple-tipped, yellow-bodied anther tubes

C_rC_r erect buds, yellow anther tubes

In the F_3 generation under discussion here, there were three plants, each having 19 chromosomes, in which no *rubra* C chromosomes were discernible; these are C 11, B 19, and C 19, in the order in which they appear in table 1. According to hypothesis all should have had yellow anther tubes and erect buds provided there were no inter-specific crossing over. Only two of the plants, however, had yellow tubes, and none of the three had erect buds. No other F_3 plants had yellow anther tubes except these two, although seven had erect buds. From the somatic garnitures it is evident that all three of these plants have one C_r longer than normal, hence as far as the cytological evidence goes the original hypothesis, that the factors for these two pairs of characters are carried in the C chromosomes of both species, has not been seriously damaged. In view of the numerous opportunities

for chromatin interchange it is evident that the classification of certain questionable chromosomes in hybrid derivatives on the basis of their resemblance to the parental chromosomes is no more than a convenience.

A genetical demonstration of crossing over involving eight homologous strands is entirely too difficult to be made with a population of only 44 plants. Furthermore, crossing over may have occurred in the F_1 as well as in the F_2 amphidiploid. If the allelomorphic pairs are considered separately, however, there is surprisingly close agreement between the observed and expected ratios for a tetraploid.

SEGREGATION FOR ANTHERTUBE PATTERN				
	Purple tubes	Tipped tubes	Yellow tubes	Total
Expected.....	1.22	41.56	1.22	44
Observed.....	2	40	2	44

SEGREGATION FOR BUD POSITION			
	Nodding buds	Erect buds	Total
Expected.....	33	11	44
Observed.....	37	7	44

If we assume that for anther tube pattern, only PPPP gives purple tubes and only pppp gives yellow tubes, and that all heterozygous constitutions are tipped, then the expected ratio of 1:34:1, very well fits the observed 2:40:2 ratio. Likewise for bud position, which is known to have a quantitative interaction in diploid derivatives, if we assume that one E in the constitution Eeee is insufficient to express dominance, then the expected genotypes will be grouped into a phenotypic ratio of 27 nodding to 9 erect. In the present instance the observed ratio of 37:7 fits fairly well, giving a probability of 83.5 per cent that a deviation of 4 is due to chance.

The two species *C. rubra* and *C. foetida* are good species from every point of view. Taxonomists of the old school, who view with disapproval the efforts of cyto-geneticists in the field of experimental evolution, seem to imagine that Mendelian character differences are not concerned in the evolution of plant species. Therefore the fact that two pairs of specific characters, such as the ones discussed above, should segregate as they do in an interspecific hybrid is good evidence that evolution is partly concerned with Mendelian factors.

THE F_4 POPULATION

This population comprised three progenies, each derived from a single F_3 plant. Two of the parents chosen were the most fertile of the F_3 plants, B 7 and B 18 (figs. 3*b* and 3*a*). The third plant, B 11 (not illustrated), was chosen because it had 21 chromosomes of the constitution $4n + A$. The number of seeds from these three progenies that germinated was about 300, and since root tips were to be fixed from all, no other F_3 plants could be included as parents for lack of convenience in their proper investigation. The three parents exhibited sufficient diversity in their chromosomes to present a fair range of the potentialities of a larger group of possible F_3 parents. The principal aims were to produce (*a*) stable derivatives of as nearly complete fertility as possible, (*b*) to note to what extent further rearrangements of chromosomes might result in further enhanced fertility, and (*c*) whether rearranged chromosome garnitures would continue in a direction already initiated, or duplicate the results observed in F_3 .

The satisfactory utilization of pollen determinations for indicating fertility in F_3 suggested a more prominent use of this study in F_4 . Incidentally a study was made of the production of pollen in several pure *Crepis* species flowering in the winter months preceding the flowering of the F_4 population. From the latter study information was secured indicating that fluctuation in the production of aborted pollen was dependent on physiological adjustments to flowering and senescence. In pure species the deviation in aborted pollen grains was extreme at the beginning of the cycle, but was shortly followed by a steady production of the amount of bad pollen expected from the genetical constitution. This, of course, would differ for different species or hybrids (Poole 1932). In order to provide a check on pollen production, therefore, two determinations of pollen percentages, three weeks apart, were made in all F_4 plants. On the whole the two determinations agreed, certain exceptions being found where the experimental error was large, but the correlation between the two determinations was of the order of 0.7208 ± 0.0388 .

In F_3 those plants showing 30 per cent or more good pollen were isolated from cross-pollination in mosquito-netting cages. As a result of this practice the caged plants were injured during a spell of several days of very hot weather, while the uncaged plants were not

affected, as the data presented in that study show. As soon as the injury was noticed the cages were removed, but this was too late to benefit any of the more highly fertile plants but one, 7.63, which was the last of these to flower.

Extreme diversity was noted among the plants of all three progenies. In rosette types alone scarcely any two plants were alike in any features, and no two were identical. Similarity was observed in only one feature which concerned the color of the setules found on the upper surface of the midribs of rosette leaves. In progeny Z 18, from B 18 of the preceding year, only five or six plants from a total of 125 reaching maturity showed any trace of color in the cell sap within the setules, all other plants being without color, regardless of whether or not the stems possessed anthocyanin. In progeny Z 7 from B 7, all the members of the progeny showed colored setules, whereas in progeny Z 11, from B 11, about half of the plants had colored and half of them uncolored setules.

A study of the degree of diversity in the population was possible for certain characters of a quantitative nature, such as percentage of good pollen, percentage of fertility, width of first flower heads when completely opened, and stature of plant when the first head flowered. This diversity is illustrated by the figures presented below for only two of these characters.

VARIABILITY OF F_4

	Percentage fertility		Percentage good pollen	
	Mean	Coef. var.	Mean	Coef. var.
All plants.....	0.603	210	15.662	61.6
Caged plants.....	1.169	103	27.641	25.8
Uncaged plants.....	0.492	253	13.303	61.7

VARIABILITY OF F_3

All plants.....	0.966	183	27.477	35.6
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Despite the injurious effects of caging, the caged plants not only exhibited a higher percentage of fertility but much less variability. The uncaged plants on the other hand showed the greatest variability and the lowest means. It will be noticed that the mean percentage of good pollen of the selected caged plants was practically the same as that for the entire F_3 population, indicating that even the choicest members of the F_4 population presented little basis for the hope of enhancing the fertility.

Since time to prepare root sections and to study the somatic chromosome garnitures of the entire 237 F_4 plants reaching maturity was not available, a sample of the entire population was selected in the following manner. The most fertile plants were selected as parents for the coming F_5 and consequently knowledge of their somatic garnitures was required. In addition to these an equal number of infertile plants were chosen for examination. Such a choice would scarcely be expected to give a true picture of the entire population as regards total chromosome numbers, because the experience in F_3 showed that fertility was practically restricted to plants possessing 20 chromosomes, whereas the less fertile plants would be expected to have as many with as without 20 chromosomes. This is approximately what was found, as may be seen below.

F_4 CHROMOSOME NUMBERS

Progeny	Parent chromosome Nos.	19	20	21	22	Total	Total progeny
Z 7	20	2	9	3	14	83
Z 11	21	1	7	3	1	12	37
Z 18	20	2	8	2	12	117
Total.....	5	24	8	1	38	237

The proportion of plants with 20 chromosomes to the total number is greater than found in F_3 where there were as many having numbers other than 20, as with 20. The disproportion observed was doubtless due to the method of selecting the plants to be examined.

The correlation of the somatic garnitures with the percentage of fertility confirmed the results obtained in F_3 , except that there were only four plants out of the 24 euploids showing a reconstituted amphidiploid garniture. In F_3 half the euploids exhibited an amphidiploid garniture. It was again observed (see table 2) that the most fertile group of derivatives was one exhibiting rearrangements in only one pair of homologues. This time, however, the information was more definite than in F_3 , for here it appears that one particular pair of homologues is more important than the others, i.e., D/E². Furthermore, a glance at the garniture formulae of F_3 and F_4 shows that the unique D chromosome from *rubra* is missing more frequently than any other chromosome. This may be shown more conveniently by the following list of missing chromosomes for F_3 and F_4 . In compiling this list it was necessary to make one assumption, which seems well justified,

TABLE 2
GENETIC AND CYTOLOGICAL DATA OF F_4
20 CHROMOSOME PLANTS

Plant No.	Per cent first week	Good pollen third week	Per cent fertility	No. of achenes	Formulae
7.70*	44.1	34.8	1.77	66	}4n
11.18	20.7	20.3	1.74	104	
11.35	4.8	0	0	
18.38	16.7	27.0	2.66	145	
7.22	10.6	9.7	0.81	26	}-C _f +C _r
7.43	19.8	21.8	0.70	13	
11.3*	7.3	8.5	0.08	3	
5*	29.8	0	0	
14	12.4	19.7	0.73	19	
18.5*	31.4	27.3	4.17	149	
11.18	8.0	5.6	0.30	8-C _r +C _f
7.21*	44.9	33.3	3.25	33	}-D+E
28*	33.0	24.3	4.46	223	
73	17.8	23.8	8.95	356	
11.17*	44.0	22.8	3.05	105	
18.47*	40.6	33.3	3.69	116	
18.28	13.7	19.0	0.77	5	}-2C _f +2C _r
71	14.4	32.9	4.62	119	
7.64	21.8	21.7	1.11	47	}-2D+2E
74*	37.2	27.4	2.53	116	
63	41.3	7.27	320-C _r +C _f -D+E
18.29	8.3	7.2	0.08	3	}-C _f +C _r -D+E
33*	33.7	30.3	1.05	34	
20	21.9	23.0	0.13	3	
				-2D+E+A

19 CHROMOSOME PLANTS

11.38	0.9	5.0	0	0-C _r
7.26	9.9	11.2	0	0-A-C _f +C _r
37	16.4	13.4	0	0-C _r -D+E
18.6	18.1	12.7	0.34	7-A-D+E
15	5.9	1.7	0	0-C _f +C _r -D

21 CHROMOSOME PLANTS

11.9	8.5	6.7	0	0	}+A
29	21.1	22.2	0.79	17	
7.30	26.3	24.2	7.58	308	}-D+2E
18.98*	41.9	28.4	2.31	54	
7.9	9.3	6.6	0.52	9-2C _f +2C _r +E
17	5.3	1.2	0	0-C _r +C _f -D+E+A
18.13	3.7	4.3	0.03	1-C _f +C _r -D+2E
11.32	6.5	0	0-2C _f +2C _r -E+D+A

22 CHROMOSOME PLANT

11.25	0	7.6	0	0-D+E+A+C _r
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*Caged plants.

viz. the inclusion of D as an extra chromosome in a euploid plant indicates a loss of its homologue, E^2 .

MISSING CHROMOSOMES

	D	E^2	C_r	C_l
F_3	20	2	11	7
F_4	22	1	5	19
	<hr/> 42	<hr/> 3	<hr/> 16	<hr/> 26

A tentative explanation of this situation is that D is the smallest chromosome and its loss is less disturbing to gametic viability. Nevertheless, no zygote is viable unless compensation for its loss is made by the substitution of its homologue E^2 , except in the case of $4n-1$ plants. This observation applies to all other chromosomes as well, for in all 20 and 21 chromosome plants examined the only viable zygotes which succeeded in reaching maturity exhibit the substitution of a homologous chromosome for the loss of any given chromosome. A total of thirteen $4n-1$ plants from both generations is a striking exception to this observation, unless we make reservations for 18.20 shown at the bottom of table 2, in which two missing D's are compensated by one E and what appeared to be one A. The parent of the progeny Z 18 was B 18 of F_3 , for which it has already been argued that the ninth A was an E with an attachment on one arm, consequently in 18.20 the extra "A" was probably a compound E^2 .

In figure 4 are illustrated three garnitures on which are shown unique chromosomes, each indicated by an arrow. This adds three more to the three already shown in figures 2a, 2b, and 3c. Such chromosomes have never been seen in the somatic cells of *C. rubra* or *C. foetida*. For obvious reasons these newly constituted chromosomes are C, D, or E, since they are the only ones in which alterations would be striking enough to lend assurance to their detection. Deficient A chromosomes would doubtless often be taken for E chromosomes, while attached E's might be taken for A's; but deficiencies in E's are represented by two examples, 18.28 and 18.98 (fig. 4a and c). No attachments to A's were noted with certainty, although several times their presence was suspected but could not be definitely identified in enough cells owing to the tendency of these longer chromosomes to twist and recurve off the equatorial plate. Plant B 9 (fig. 3c) has already been referred to as illustrating one

satellited C_r too long for a normal chromosome, and one unsatellited C_r too short to be normal. Other examples have already been mentioned, especially when discussing the genetic evidence for anther tube pattern and bud position. Here the presence of longer than normal C_r chromosomes was pointed out, although the plates are not

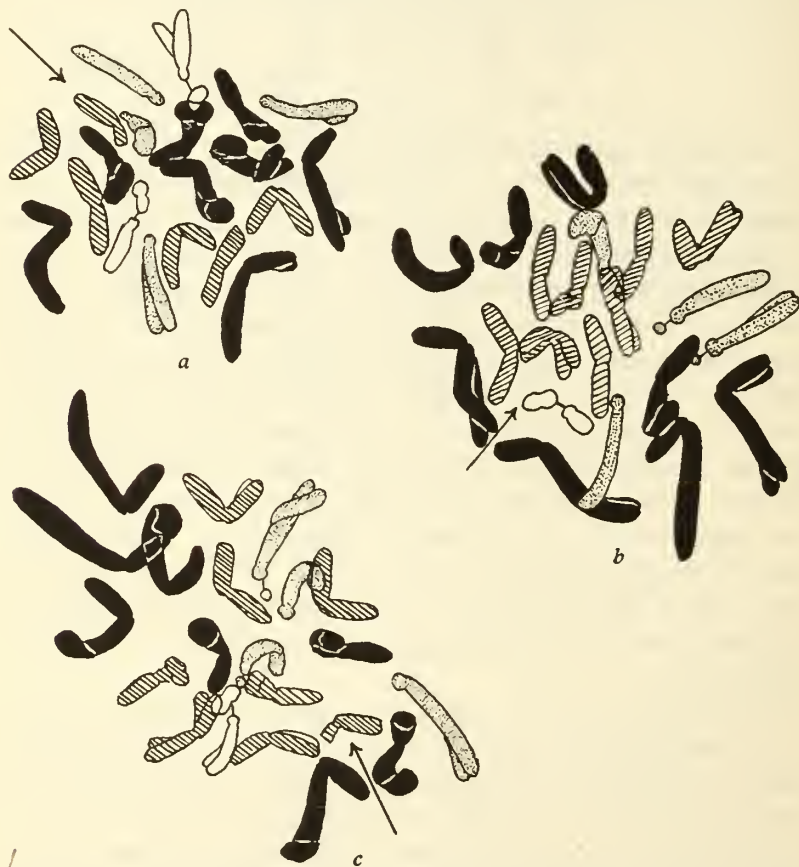


Fig. 4. Metaphase figures from three F_4 plants each showing a deficient chromosome: (a) 18.28, a euploid with a deficient E, formula $-2C_r+C_r$; (b) 7.30, an aneuploid with a deficient D; formula $-D+2E$; (c) 18.98, an aneuploid with a deficient E, formula $-D+2E$.

illustrated in the included drawings. The most readily detected alterations are those occurring on D's, three included illustrations showing a deficiency (fig. 4b), an attachment to the body of the chromosome (fig. 2a), and a loss of the satellite in a plant possessing 3 D's, the other two of which were entirely normal (fig. 2b).

In view of the generally random nature of chromosome assortment in these derivatives, it is not surprising that F_3 plants like B 7

and B 18, each of which lacked one D chromosome, should have produced progeny possessing two D's. This situation was observed five times out of a total of fourteen plants in progeny Z 7, and four times in progeny Z 18 out of a total of twelve plants.

Fertility.—In both F_3 and F_4 fertility is practically restricted to plants possessing the euploid chromosome number which, as we have seen, is maintained in the majority of cases not through a transmitted or reconstituted amphidiploid garniture, but as a result of rearrangements often quite complicated. It is remarkable, then, that the less complex rearrangements produce the more fertile combinations. This is most evident when rearrangements involve the single homologous pair D/E^2 . It is also seen from table 2, plant 7.63, where a second pair is involved, and also in F_3 in B 18 where two pairs were involved, one of them in all cases being D/E^2 . The influence of D/E^2 is especially noticeable in the only two F_4 aneuploid plants exhibiting fertility, 7.30 and 18.98 (figs. 4*b* and 4*c*) both of which have twenty-one chromosomes and the formula $4n - D + 2E$. In these two plants there is an extra E chromosome but at the same time there is one deficient chromosome in each plant. Because of the deficiency the nuclear balance has not been greatly upset by the addition of a single E chromosome.

The three most fertile plants of this population are three plants which were not caged, because in two of them, 7.73 and 7.30, the first pollen determination failed to show 30 per cent or more of good pollen grains. Although 7.63, the third most fertile plant, did show over 30 per cent good grains, it was the plant mentioned above which had its cage removed when the injury caused by overheating was first noticed. All caged plants which were studied for their somatic garnitures are indicated in table 2 with an asterisk, and it can be seen that their high pollen counts promised a higher degree of fertility than was observed. In the study of the pollen grains in relation to fertility, previously alluded to, it was shown that the caged plants as a group were the only plants to show no correlation between good pollen and fertility.

DISCUSSION

There have now been reported in the literature of the past six years an aggregate of more than twenty experimentally verified amphidiploid hybrids, some of which were known as far back as 1880, although their true nature was not understood. Some of the investigators fail to report on fertility, but most of them have reported as to the regularity or irregularity of meiosis. From the list there appear to be ten which are as fertile as good species:

1. *Anemone sylvestris* x *A. magellanica* (Janczewski 1889) ?
2. *Aesculus hippocastanum* x *A. pavia* (Skovsted 1929)
3. *Fragaria bracteata* x *F. helleri* (Ichiyama 1926)
4. *Raphanus sativus* x *Brassica oleracea* (Karpechenko 1928)
5. *Rosa Wilsoni* (Blackburn and Harrison 1924)
6. *Nicotiana glauca* (Clausen & Goodspeed 1925)
7. *Nicotiana tabacum* x *N. rustica* (Rybin 1927)
8. *Aegilops ovata* x *Triticum dicoccoides* (Tschermak and Bleier 1926)
9. *Spartina townsendii* (Huskins 1931)
10. *Primula kewensis* (Newton and Pellew 1929)

In view of the fact that Janczewski's probable amphidiploid was not examined cytologically, there may be some question raised as to its inclusion in the species-like group. Certain others, now to be considered, are only fairly fertile, or show some amount of segregation in their progeny. Many, however, were reported to be highly irregular, or even entirely sterile:

11. *Digitalis ambigua* x *D. purpurea* (Buxton and Newton 1928)
12. *Triticum vulgare* x *Secale cereale* (Levitsky and Benetzkaja 1929)
13. *Nicotiana paniculata* x *N. rustica* (Lammerts 1931)
14. *Solanum nigrum* x *S. luteum* (Jorgensen 1928)
15. *Crepis capillaris* x *C. dioscoridis* (Babcock & Navashin 1930)
16. *C. capillaris* x *C. tectorum* (Hollingshead 1930)
17. *C. rubra* x *C. foetida* (Poole 1931)
18. *Saxifraga potternensis* (Marsden-Jones 1930)

In the group 11-18, five are reported to show the presence, with variable frequency, of quadrivalent chromosomes in meiosis, and one other occurs among the list in which a tetraploid plant appearing in one of the parent species showed quadrivalents, although the number of pollen mother cells examined in the amphidiploid was insufficient to show whether or not the phenomenon existed (Jorgensen 1928). Despite the lack of detailed data reported along with the announce-

ments, it is clear that there are many different kinds of amphidiploids as to behavior; and certainly several different categories of origin have been reported.

The presence of quadrivalent chromosomes in species hybrids raises a question of correct terminology. Viewed solely from the standpoint of cytology, objections could be raised against calling any hybrid an amphidiploid if quadrivalents were observed. In such a case it could be argued that the occurrence of quadrivalents denotes a tetraploid rather than an amphidiploid condition. On the other hand, as soon as the taxonomic view is considered a new complexion is put on the matter. If both parents were unquestionably good species, possessing numerous character contrasts, in addition to obviously distinct somatic garnitures, then a $4n$ hybrid between two such species is undeniably an amphidiploid. This question at least shows the desirability of considering the matter from more than one viewpoint.

Those amphidiploids which behave with the regularity of good species are undoubtedly representative of types which have functioned in producing genera wherein most of the species exhibit chromosome numbers in arithmetical series. Some genera appear to have evolved exclusively by this method, whereas others show only occasional species which probably originated through amphidiploidy. Although all of them are characterized by the possession of a complete diploid complement from both parents of the preceding generation, they have had different methods of origin, and exhibit different classes of behavior. Among the different ways in which these hybrids originated may be noted two main methods, (a) non-reduction of gametes, and (b) somatic duplication. Most of them have been reported to originate through the non-reduction of gametes, but among those originating by somatic duplication three subcategories exist: (a) bud variation, as in *P. kewensis*, (b) callose shoots, as in *S. nigrum* \times *S. luteum*, and (c) endoduplication, as in *Nicotiana digluta*.

The evidence of quadrivalent chromosomes in five or six of the amphidiploids in the above list, and especially in *rubra-foetida*, makes it clear that much of the meiotic irregularity observed is due to genetic and cytological homology between the parent species. Such homology undoubtedly delays or altogether prevents the operation of amphidiploidy as an agency in evolution. Where homology is at a minimum, rearrangement of chromosome complements leading to the formation of harmonious reaction systems is ultimately possible and

will occur, as indicated by the tendency of *Nicotiana rustica-paniculata* in this direction. On the other hand, where homology is at a maximum, perhaps no amount of rearrangement will avoid disjunctional irregularities. Hence there are at least three kinds of amphidiploids possible: (a) no interspecific pairing, with regular maturation divisions; (b) a minimum amount of interspecific pairing, with rearrangement products leading to stable derivatives; and (c) a maximum amount of interspecific pairing, with probably no rearrangement products.

In the present state of this investigation it is difficult to say how the *rubra-foetida* hybrid will eventually behave. It is clear that thus far little ground exists for believing that stability will be reached. The hope that selection of the most highly fertile plants would result in the isolation of strains with gradually increasing fertility appears, from existing evidence, to be abortive. The garniture rearrangements of F_3 which were most fertile continued to assort at random in F_4 , producing some of the same types, as well as many others, none of which produced any improvement in percentage of good pollen. Furthermore, in both generations the maximum fertility occurred only in euploid plants, and in such a way as to suggest restriction of fertility to a very narrow departure from the amphidiploid garniture. This departure was, in all the more fertile cases, of an unbalanced nature. In general, if a chromosome were missing from the $4n$ set, the evidence indicated that the gamete involved had been fertilized by a gamete bearing an extra interspecific homologue, otherwise zygotic lethality resulted. This was true of all plants, euploids and aneuploids. But in $4n-1$ aneuploids there was necessarily one, although only one, uncompensated loss, regardless of other rearrangements, and in $4n+1$ aneuploids there was one, and only one, extra homologue. There were two $4n+2$ plants, one in each generation, B 21 and 11.25. In both cases there were *two* extra homologues regardless of other rearrangements.

In view of this evidence the limits of gametic and zygotic viability may be inferred. From the general nature of the evidence it is probable that viable gametes carried only the numbers $2n$, and $2n$ plus or minus 1 chromosome.

The evidence for zygotic viability is more definite. In *euploids* zygotic viability is apparently limited by (a) the necessity of a missing chromosome of a $2n-1$ gamete meeting an extra homologue of the same set in a conjugating gamete, which must be $2n+1$; or (b) by

gametes with the number $2n$, but with an extra chromosome of one set and a missing chromosome of another meeting a gamete with a missing chromosome of the first set and an extra chromosome of the second set.

In *aneuploids* the same compensation is required, as shown by the evidence when rearrangements have involved more than one homologous set. The difference in zygotic viability between euploid and aneuploid plants, however, is that in aneuploids of the type $4n-1$ one member of a given set is missing and in the type $4n+1$ one member of any set is extra, all other losses being compensated. In $4n+2$ plants one member of any two sets may be extra or missing, although no $4n-2$ plants have yet been observed.

In regard to zygotic fertility rather definite conclusions may be drawn also, for the most fertile zygotes of both generations were those in which rearrangements involved a lack of just one D chromosome, which represents the loss of the smallest possible amount of chromatin material. This quantitative feature of chromatin loss is emphasized by the fact that the two fertile $4n+1$ plants, 7.30 and 18.98, had chromosomal deficiencies which balanced the addition of one extra chromosome.

Irregularities of the type occurring in *Pisum*, *Oenothera*, and *Datura* are still possible here in view of the discovery of newly constituted chromosomes, as illustrated in figures 2a, 2b, 3c, 4a, 4b, and 4c. Furthermore, we have here evidence of the kind of chromosomal changes which differentiate the majority of *Crepis* species from one another. The majority of *Crepis* species have the same chromosome number, four pairs. Yet the species are all good taxonomically, and in addition to their factorial differences, most of them show slight alterations in shape or size of homologous chromosomes, of the kind here considered.

In view of these facts, investigations of *rubra-foetida* amphidiploid derivatives will be continued with progenies of large numbers. Either stable derivatives will eventually be found, or they will not. In case they are found the advantages of small chromosome numbers, and a certain amount of reliable chromosome individuality, will throw light on important evolutionary problems common in the plant kingdom—advantages not found in just this combination in less favorable material.

Even though no stable derivatives are eventually found from the amphidiploid here investigated, this will not invalidate the hypothesis

that the American species of *Crepis*, having a base number of $n=11$, originated through amphidiploidy. As has been pointed out (Babeock and Navashin, 1930) the assumed amphidiploid hybrids most probably originated from crosses between species having $n=4$ and $n=7$. All of the known species with these chromosome numbers are sufficiently distant in their relationship that no meiotic irregularities would prevent full fertility in their amphidiploid hybrids.

SUMMARY

1. In the first selfed generation of derivatives from an amphidiploid plant of *C. rubra* x *C. foetida* the chromosome numbers were roughly one-half euploid and one-half aneuploid. The aneuploids were equally divided between $4n-1$ and $4n+1$, whereas the euploids were equally divided between those having no rearrangements in the $4n$ set, and those exhibiting various degrees of rearrangement from that set.

2. The second selfed generation from three plants of the first generation was not completely examined cytologically, but those examined indicated that the range in chromosomal distribution was analogous to that found in the first. Since none of the three parents was amphidiploid in garniture, however, the number of reconstituted amphidiploids was not as high as before, but the fact that they existed is indicative of the random nature of chromosome assortment.

3. Fertility in both selfed generations was almost entirely restricted to euploid plants, and among these it was especially marked in plants having chromosomal rearrangements involving but one pair of interspecific homologues, D/E². There was no tendency in plants having garnitures that were completely *rubra* or completely *foetida* for any homologous sets to be fertile.

4. The only aneuploids exhibiting any degree of fertility were two having rearrangements involving the same set of homologues, D/E, and these were both of the formula $4n-D+2E$. Furthermore, these two fertile $4n+1$ plants each had, in addition to one extra chromosome, one deficient chromosome, thus balancing the additional amount of chromatin matter.

5. The more fertile plants in both generations had garnitures of an unbalanced nature, even in euploids. But these most fertile deriva-

tives displayed very low fertility, never exceeding 10 per cent, which indicates that stable races are not likely to be derived from this amphidiploid.

6. The evidence for all plants, euploid or aneuploid, suggested that viability of the zygote was contingent on the compensatory presence of the interspecific homologues of any of the chromosomes lacking from one of the pairing gametes. Never was the absence of a particular chromosome compensated by the additional presence of a non-homologue.

7. In $4n-1$ aneuploids there was one, but only one, uncompensated loss; all other losses from one gamete being compensated by the presence of an extra interspecific homologue in the conjugating gamete. In $4n+1$ or $+2$ plants extra homologues were present, but no losses were uncompensated.

8. The interspecific allelomorphie character pairs noddling-erect buds and purple-yellow anther tube patterns apparently segregated in the tetraploid ratio, even in the limited population available, corroborating the cytological evidence of random assortment.

9. Several well demonstrated cases of newly constituted chromosomes were observed in both selfed generations.

LITERATURE CITED

- BARCOCK, E. B., and CLAUSEN, J.
1929. Meiosis in two species and three hybrids of *Crepis*. Univ. Calif. Publ. Agr. Sci., 2:401-432.
- BARCOCK, E. B., and NAVASHIN, M.
1930. The Genus *Crepis*. Bibliographia Genetica, 6:1-90.
- BELLING, J., and BLAKESLEE, A. F.
1924. The distribution of chromosomes in tetraploid *Daturas*. Am. Nat. 58:60-70.
- BLACKBURN, K. B., and HARRISON, J. W. H.
1924. Genetical and Cytological studies in hybrid roses. Brit. Jour. Exp. Biol., 1:557-570.
- BUXTON, B. H., and NEWTON, W. C. F.
1928. Hybrids of *Digitalis ambigua* x *D. purpurea*. Jour. Gen., 19:269-279.
- CLAUSEN, R. E., and GOODSPEED, T. H.
1925. Interspecific Hybridization in *Nicotiana*. II. A tetraploid *glutinosa-tabacum* hybrid. Genetics, 10:278-284.
- HOLLINGSHEAD, E. L.
1930. Hybrids of *Crepis capillaris* x *C. tectorum*. Univ. Calif. Publ. Agr. Sci., 6:55-95.
- HUSKINS, C. L.
1931. The Origin of *Spartina townsendii*. Genetica, 13:548-555.
- ICHIYAMA, K.
1926. Cytological and Genetical studies on *Fragaria*. Genetics, 11:590-604.
- JANCZEWSKI.
1889. *Anemone sylvestris* x *A. magellanica*. Bull. Acad. Cracovie, June 1889: 24 and *ibid.*, 1892:228.
- JORGENSEN, C. A.
1928. The Experimental formation of heteroploid plants in *Solanum*. Jour. Gen. 19:133-210.
- KARPECHENKO, G. D.
1928. The Production of Polyploid gametes in hybrids. Hereditas, 9:349-368.
- LAMMERTS, W. E.
1931. Interspecific Hybridization in *Nicotiana*. XII. The amphidiploid *rustica-paniculata* hybrid. Genetics, 16:191-211.
- LEVITSKY, G. A., and BENETZKAIA, G. K.
1929. Cytological investigations of constant intermediate Rye-Wheat hybrids. Proc. U.S.S.R. Con. Gen. 2:345-352.
- MARSDEN-JONES, E. M., *et al.*
1930. History of a tetraploid Saxifrage. Jour. Gen., 23:83-121.
- NEWTON, W. C. F., and PELLEW, C.
1929. *Primula kewensis* and its derivatives. Jour. Gen., 20:403-467.

POOLE, C. F.

1931. The interspecific hybrid *Crepis rubra* × *C. foetida*, and some of its derivatives. I. Univ. Calif. Publ. Agr. Sci., 6:169-200.

1932. Pollen grain studies as an indication of fertility in hybrids. MS.

RYBIN, V. A.

1927. Polyploid hybrids of *N. tabacum* × *N. rustica*. Bull. Ap. Bot. and Gen., 17:191-240.

1929. Ueber einen allotetraploide Bastard von *N. tabacum* × *N. sylvestris*. Deutsche Bot. Gesell., 47:385-395.

SKOVSTED, A.

1929. Cytological investigations of the genus *Aesculus*. Hereditas, 12:64-70.

TSCHERMAK, E., and BLEIER, H.

1926. Ueber fruchtbare *Aegilops*-Weizenbastarde. Bericht, Deutsche Bot. Gesell., 44:110-132.

